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Neotypification of *Claviceps humidiphila* and recognition of *C. bavariensis* sp. nov.

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Abstract—*Claviceps humidiphila* [≡ *C. purpurea var. phalaridis*] was previously typified with the holotype collected by Tanda in Japan and an epitype from Bavaria, Germany. Phylogenetic analyses based on translation elongation factor 1-α (TEF1-α) and RNA polymerase II second largest subunit (RPB2) indicated that the previously designated epitype from Germany was not conspecific with the Japanese species. The German specimen is proposed as a new species, *C. bavariensis,* and a specimen collected from the type location (Chiba, Japan) is designated as a neotype for *C. humidiphila*, replacing the lost holotype.

Key words—*Ascomycota*, *Clavicipitaceae*, ergot fungi, *Hypocreales,* taxonomy

Introduction

Claviceps was validly published by Tulasne (1853), as discussed by Donk (1963). *Claviceps purpurea* Tul. was selected as lectotype by Clements & Shear (1931) and accepted by Donk (1963). Tulasne (1853) noted the hosts of *C. purpurea* include cereal crops (e.g., *Avena*, *Secale*, *Triticum*) and grasses (e.g., *Alopecurus*, *Dactylis*, *Lolium*, *Poa*). Thereafter, the reported host range increased to more than 400 species in *Poaceae*. Molecular studies (Pažoutová & al. 2000, Douhan & al. 2008) revealed cryptic speciation within the pre-molecular concept of *C. purpurea* s.lat. Pažoutová & al. (2015) provided

formal taxonomic descriptions for four phylogenetic species to replace *C. purpurea* s.lat.: *C. purpurea* s.str., *C. arundinis*, *C. humidiphila*, and *C. spartinae*.

Claviceps humidiphila was characterized by its frequently larger conidia than *C. purpurea*, sclerotia capable of floating on water, and typical habit on *Calamagrostis*, *Deschampsia caespitosa*, and *Phalaris arundinacea*. Previously, Tanda recognized three varieties: *C. purpurea* var. *alopecuri*, *C. purpurea* var. *dactylidis*, and *C. purpurea* var. *phalaridis*, producing conspicuously larger conidia and on specific host plants. *Claviceps purpurea* var. *alopecuri* was found on *Alopecurus*, but also on *Agrostis clavata*, *A. palustris*, *Calamagrostis epigejos*, *C. hakonensis*, *Holcus lanatus*, *Phleum pratense*, *Poa* spp., *Polypogon fugax*, and *Trisetum bifidum* (Tanda 1978a,b,c, 1979a,c, 1981; Tanda & Kawatani 1980). *Claviceps purpurea* var. *dactylidis* was found on *Dactylis glomerata* and *Calamagrostis epigejos* (Tanda 1980). *Claviceps purpurea* var. *phalaridis* occurred on *Phalaris*, *Calamagrostis epigejos*, and *C. pseudophragmites* (Tanda 1979a).

Pažoutová & al. (2015), who noted that the conidial dimension and host range of *C. purpurea* var. *dactylidis* and *C. purpurea* var. *alopecuri* overlapped with both *C. purpurea* s.str. and *C. humidiphila*, treated the varieties as taxa of uncertain identities. However, *C. purpurea* var. *phalaridis* was considered as representing the same taxon as the species later named *C. humidiphila*. The name *C. purpurea* var. *phalaridis* could not be elevated to species rank as a stat. nov. because this would create an illegitimate later homonym of *C. phalaridis* J. Walker [≡ *Aciculosporium phalaridis* (J. Walker) M. Kolařík & Píchová]. Instead, *Claviceps humidiphila* was proposed as a nom. nov. based on the replaced synonym *Claviceps purpurea* var. *phalaridis*. Tanda (1979b) designated a holotype from Chiba, Japan, now believed to have been lost (see Taxonomy: Comments below, p. 78). Pažoutová & al. (2015) designated an epitype from Bavaria, Germany, but phylogenetic analysis indicates that the epitytpe is not conspecific with Japanese collections. Here, we propose *Claviceps bavariensis* as a new species holotypified by the Bavarian collection and designate a recent specimen from Chiba, Japan, as the neotype of *C. purpurea* var. *phalaridis* [≡ *C. humidiphila*].

Material & methods

DNA extraction, PCR, sequencing, analyses

Axenic cultures were developed for two samples collected from Canada (Alberta) and gDNA extracted following Shoukouhi & al. (2019). For 29 samples from Japan

(Chiba, Gifu, Ishikawa, Kanagawa, Miyagi, Nagano, Niigata, Tochigi, Toyama), axenic cultures were developed from peeled ergots that were surface-disinfected in 2% sodium hypochlorite solution for 1 min; gDNA was extracted according to Izumitsu & al. (2012). Two genomic regions, RPB2 (RNA polymerase II second largest subunit) and TEF1-α (translation elongation factor 1-α), were amplified and sequenced using modified fRPB2-5F (5′-TTTCGTGGTATTGTTCGCAGA-3′) specific for ergot fungi (Pažoutová & al. 2015) and fRPB2-7cR (Liu & al. 1999), and the primer pair EF1-983F and EF1-2218R (Rehner & Buckley 2005). For 29 samples from Japan, the RPB2 regions were amplified and sequenced using Cla-RPB2 (5′-CAGTGAAACCAGAAAGGCCTTC-3′) and modified fRPB2-7cR (5′-CCCATGGCCTGCTTACCCAT-3′). Polymerase chain reactions (PCRs) were performed in 10 µL volumes containing final concentrations of 10× Titanium Taq buffer (with 3.5 mM $MgCl_2$), 0.1 mM dNTPs, 0.08 µM each of forward and reverse primer, 50× Titanium Taq polymerase (Takara Bio, California), 0.01 mg BSA, and 1 µL of DNA template. A touchdown protocol was applied with an initial denaturation at 95 °C for 3 min, followed by 5 cycles of 95 °C for 1 min, annealing at 63 °C (decrease 1 °C per cycle) for 45 s, and extension at 72 °C for 1 min 30 s followed by 30 cycles of 95 °C for 1 min, annealing at 58 °C for 45 s, extension at 72 °C for 1 min 30 s, concluding with a final extension at 72 °C for 8 min. PCR products were sequenced using a ABI BigDye Terminator 3.1 cycling sequencing kit on Applied Biosciences Prism 3130xl Genetic Analyzer.

Fifty-nine reference RPB2 and TEF1-α sequences from previous studies downloaded from GenBank were compiled and aligned with sequences generated in the present study (31 strains) using MAFFT online version 7 (Katoh & al. 2019, https://mafft.cbrc.jp/alignment/server/, accessed in Aug 2020). Alignments of concatenated sequences were generated in Geneious Prime 2020.1.2. (http://www.geneious.com). Maximum parsimony (MP) analyses were performed using PAUP* 4.0b10 (Swofford 2002). Heuristic searches with 200 replicates of random stepwise addition and tree bisection-reconnection branch swapping were conducted with a limit of 1,000,000 re-arrangements for each replicate. Bootstrapping analyses used 2000 replicates of a full heuristic search with random stepwise addition of 20 replicates with limit of 50,000 rearrangements per replicate. Bayesian inference analyses were conducted using MrBayes 3.2 (Ronquist & al. 2012) with a GTR model chosen by a previous study (Liu & al. 2021). Each run was set to four chains of 100,000,000 MCMC generations, sampling frequency was every 2000 generations, terminated when the standard deviation of the average split frequency was lower than 0.01. The BI consensus tree was directly generated after 25% burn-in.

Morphological examination

Specimens were conserved in the Herbarium, Department of Botany, National Museum of Nature and Science, Tsukuba, Japan (TNS) and the Canadian National Mycological Herbarium (DAOM); cultures were conserved in NARO Genebank,

Microorganism Section, Genetic Resources Center, National Agriculture and Food Research Organization, Tsukuba, Japan (MAFF).

Sclerotia were tested for floating ability using the protocol developed by Pažoutová & al. (2000). The shape, size, color, and surface of sclerotia were recorded from observations of available sclerotia. Specimens were photographed using a DFC425 camera and Leica Application Suite 4.12.0 software attached to Leica M165C or Motic SMZ-168-BL stereo microscopes. Colors were characterized using html color HEX codes at the maximum approximation (https://htmlcolorcodes.com/color-names/). Sclerotial tissues were sectioned by hand for microscopical examination. Conidia were washed off from sclerotial surfaces and mounted in water or lactic acid for examination using a Zeiss Axio Scope.A1 microscope with differential interference contrast (DIC) illumination, or Zeiss Imager M2. Microphotographs were taken with either a Jenoptik ProgRes SpeedXTcore 5 digital camera using ProgRes image processing software for CCC samples or with (alternatively) an Axiocam 503 color camera using a Zeiss ZEN (blue edition) 2.6 pro imaging processing for Canadian samples, and for Japanese samples a MicroPublisher 5.0 or 3.3 RTV using QImaging QCapture Pro software or a WRAYCAM-NOA2000 using the Wraymer MicroStudio processing package. Conidiogenesis in sclerotia was observed under a Zeiss Imager M2 by mounting small fragments of internal sclerotial tissue obtained by slicing or picking.

Germinated sclerotia were obtained by chill-treating sclerotia on moistened quartz sand in sealed cups at 4 °C about 3 months followed by incubation under 12 h light/12 h dark condition at 20 °C for a few weeks. Ascostromal sections were prepared as described by Tanaka & al. (2020). Examination and microphotography of asci and ascospores followed the same procedures as used for conidia.

Phylogenetic results

DNA sequences generated in this study were submitted to GenBank. Including 59 reference sequences, the TEF1-α and RPB2 matrices comprised 90 taxa and 918 and 1027 characters. The phylogenetic tree based on the concatenated alignment of TEF1-α and RPB2 placed the specimen collected from the type location of *C. purpurea* var. *phalaridis* (TNS-F-60506 on *Phalaris arundinacea* Chiba) in a different clade, sister to CCC 434 (= DAOMC 251717), the epitype from Germany. Here, we retain the name *C. humidiphila* for the Japanese clade and propose *C. bavariensis* for the German clade. The two are sister species and closely related to *C. arundinis* and *C. perihumidiphila* (Fig. 1). Phylogenetic trees based on the individual genes showed localized low resolution as discussed by Shoukouhi & al. (2019 and Liu & al. (2020).

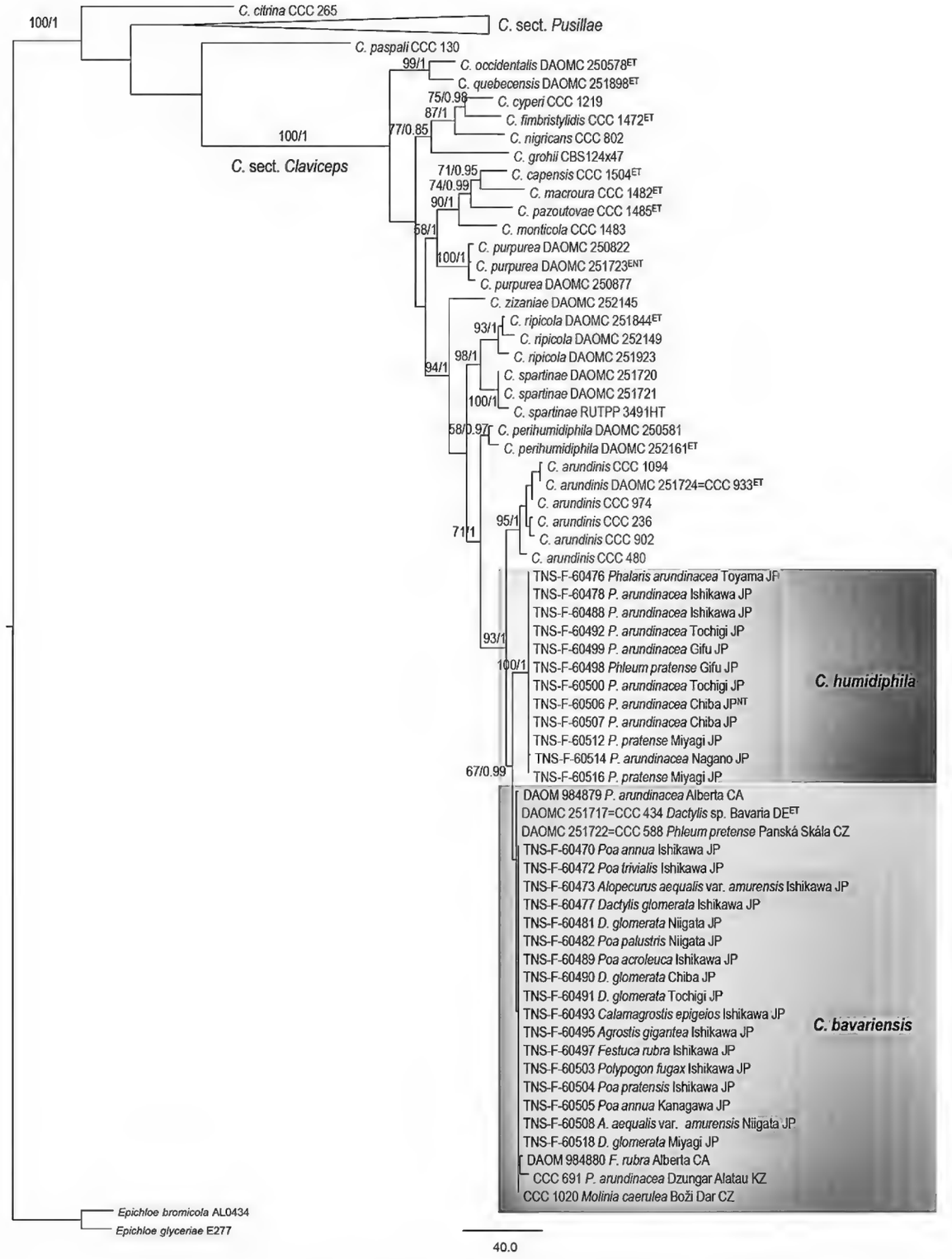

FIG. 1. Phylogenetic tree based on the concatenated sequence alignments of TEF1-α and RPB2 showing the separation and close relationship of *Claviceps humidiphila* and *C. bavariensis*. Reference sequences are in gray font, showing species names and voucher numbers; 24 taxa in *C.* sect. *Pussillae* are abbreviated to a single branch. The labels of the sequences in clades *C. humidiphila* and *C. bavariensis* include voucher numbers, hosts, locations, and country abbreviations: CA Canada; CZ Czech Republican; DE Germany; JP Japan; KZ Kazakhstan. Superscripts ET = ex-type, NT = neotype. Values on branches are bootstrapping values for MP analyses/posterior probability of BI.

Taxonomy

Claviceps humidiphila Pažoutová & M. Kolařík,
Fungal Biol. 119(1): 22 (2015) Fig. 2

≡ *Claviceps purpurea* var. *phalaridis* Tanda, J. Agric. Sci. (Tokyo) 24: 84 (1979).

Holotype (**lost**)—Japan, Chiba, Chiba, Yukijirushi Farm on *Phalaris arundinacea*, Aug 1, 1969, Seinosuke Tanda (TUAMH-PA 921).

Neotype (**here designated**, MBT395372)—Japan, Chiba, Chiba, Inage [= the holotype locality], 35.6672°N 140.1353°E, on *Phalaris arundinacea*, June 23, 2017, Eiji Tanaka (**Neotype**, TNS-F-60506; ex-type culture MAFF 247310; GenBank LC598958, LC598987).

Sclerotia dark purple (#1C0336) to black (#05000A), 4–15 × 0.8–1.4 mm, cylindrical, floating in fresh water. Conidia from sclerotial surface hyaline, aseptate, allantoid, oblong, ovoid, ellipsoid, lunate, 6.0–16.9 × 3.6–5.9 µm, L/W ratio 2.3–2.6.

Ascostromata light orange (#E2C86C) to purplish red (#E6B0AA). Stipe filiform, 6–13 mm long, 0.1–0.4 mm wide. Capitellum 0.5–1.9 mm diam. Perithecia obovoid, slightly fusiform to pyriform, 154–314 × 100–157 µm. Asci cylindrical 50–110 × 2.5–5.5 µm. Ascospores filiform, 85–110 µm long.

Additional specimens examined—**JAPAN**, **Chiba**, **Sakura**, Kodake, 35.7388°N 140.1661°E, on *Phalaris arundinacea*, June 23, 2017, Eiji Tanaka (TNS-F-60507; GenBank LC598959, LC598988), (TNS-F-60525, fruiting bodies derived from TNS-F-60507); **Gifu**, **Takayama**, Hirayu, 36.1917°N 137.5510°E, on *P. arundinacea*, Aug 14, 2016, Kazuhito Tanada (TNS-F-60499; GenBank LC598963, LC598992); 36.1914°N 137.5503°E, on *Phleum pratense*, Aug 14, 2016, Kazuhito Tanada (TNS-F-60498; GenBank LC598967, LC598996); **Ishikawa**, **Nomi**, Nabetani, 36.4189°N 136.5575°E, on *P. arundinacea*, June 30, 2016, Eiji Tanaka (TNS-F-60488; GenBank LC598961, LC598990); **Uchinada**, Kose, 36.6936°N 136.6842°E, on *P. arundinacea*, June 18, 2016, Eiji Tanaka (TNS-F-60478, MAFF 247302; GenBank LC598960, LC598989), (TNS-F-60524, fruiting bodies derived from TNS-F-60478); **Miyagi**, **Ohira**, 38.4683°N 140.8833°E, on *P. pratense*, Aug 10, 2017, Kazuhito Tanada (TNS-F-60512; GenBank LC598968, LC598997); **Nagano**, **Nagano**, Togakushi, 36.7711°N 138.0900°E, on *P. arundinacea*, Aug 1, 2018, Eiji Tanaka (TNS-F-60514; GenBank LC598966, LC598995); **Tochigi**, **Tochigi**, Fujioka, 36.2711°N 139.6567°E, on *P. arundinacea*, July 6, 2016, Kazuhito Tanada (TNS-F-60492, MAFF 247305; GenBank LC598965, LC598994); Nikko, Okukinu, 36.8681°N 139.3947°E, on *P. arundinacea*, Aug 15, 2016, Kazuhito Tanada (TNS-F-60500, MAFF 247310; GenBank LC598964, LC598993); **Toyama**, **Nanto**, Fukumitsu, 36.5636°N 136.8778°E, on *P. arundinacea*, June 16, 2016, Eiji Tanaka (TNS-F-60476, MAFF 247301; GenBank LC598962, LC598991).

Hosts—*Phalaris*, *Phleum*

Comments—In the original description of *C. purpurea* var. *phalaridis* (Tanda 1979b), the specimens TUAMH-PA 820 and TUAMH-PA 921 were listed as the first and second specimens examined followed by the word "Holotype." It is

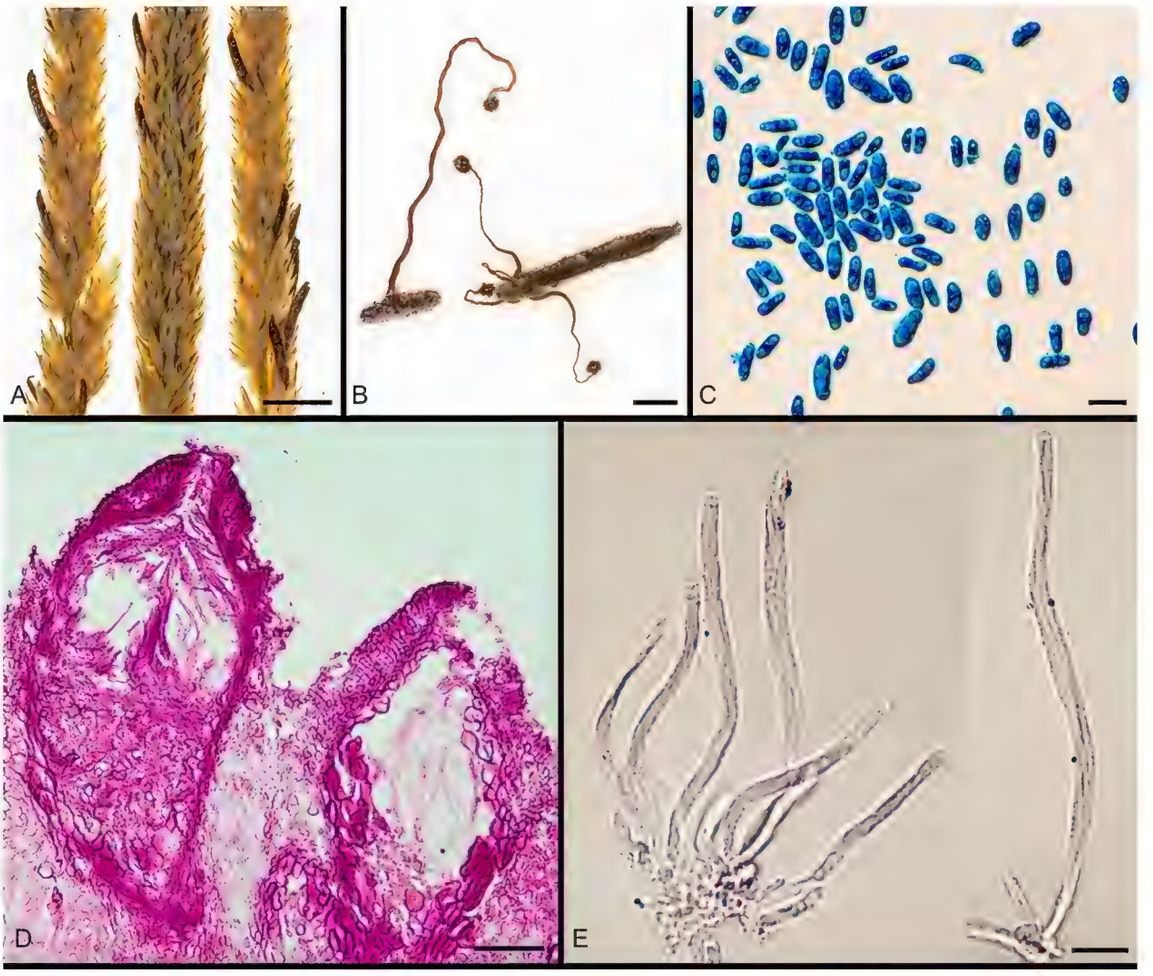

Fig. 2. *Claviceps humidiphila*: A. Sclerotia on *Phalaris arundinacea* (TNS-F-60478); B. Ascostromata (TNS-F-60524) developed from germinated sclerotia (TNS-F-60478); C. Conidia washed off sclerotia (TNS-F-60478); D. Perithecia semi-embedded in stromata; E. Asci with thick caps and filiform ascospores (TNS-F-60524). Scale bars: A = 10 mm; B = 2 mm; D = 50 µm; C, E = 10 µm.

likely that TUAMH-PA 921 was designated as holotype, although PA 820 was recorded as holotype in Pažoutová & al. (2015), possibly because PA 921 was erroneously overlooked. Nevertheless, we searched all of Tanda's research collections (1979b) using several approaches. Firstly, the abbreviation TUAMH is not cited in Index Herbariorum (http://sweetgum.nybg.org/science/ih/) where TUAT is cited as the correct code for Tokyo University of Agriculture Herbarium (Museum); however, TUAT is currently inactive, and its 100,000 specimens were transferred to the National Museum of Nature and Science, Tokyo (TNS) in 2007. We searched the TNS online database (http://db.kahaku.go.jp/webmuseum_en) for *Claviceps purpurea* collected in Japan; of the 18 specimens located, none had been collected from Chiba or

related to TUAMH PA 820 or PA 921. Next, we contacted the herbarium director and curator (Dr. Masanobu Higuchi and Dr. Tsuyoshi Hosoya), who recommended contacting Dr. Keiichi Motohashi at Tokyo University directly. According to a personal communication with Dr. Seinosuke Tanda, Tanda left all his un-submitted specimens in the laboratory at Tokyo University of Agriculture, where Dr. Motohashi checked 6000 specimens that had been collected by Dr. Tanda; 90% represented powdery mildews and the rest were rust fungi; no *Claviceps* specimens were found, possibly because other specimens had been discarded. Based on the collective evidence, it is likely that the specimens linked to the name *C. purpurea* var. *phalaridis* were lost. Therefore, we hereby designate a new specimen from the type location as neotype.

According to Tanda (1979b), the sclerotia were of different sizes. One specimen collected from Asahi-mura, Niigata (PA007), possessed much shorter sclerotia (2.3–7.7 × 0.6–1.3 mm; n = 215), while others had a wider size range (2.2–15.4 × 0.5–1.5 mm; n = 599). The number of stromata developed from sclerotia varied from 1 to 11, for which Tanda (1979b) cited the dimensions of 0.1–1.6 × 0.2–1.9 mm (capitellum), 2–13 × 0.1–0.9 mm (stipe), and 4.4–16.9 × 2.4–5.9 µm [L/W 2.2–2.6] (conidia; n = 1350). Our sclerotial, stromatal, and conidial measurements match those cited by Tanda. In addition, Tanda (1979b) also recorded perithecia at 161–217 × 91–130 µm (a slightly narrower range than our data), asci at 74–133 × 1.8–4.2 µm (slightly longer than our data), and ascospores at 72–129 µm (similar to our data).

Claviceps bavariensis M. Kolařík, E. Tanaka & M. Liu, **sp. nov.** Fig. 3

MB 838352

[≡ "*Claviceps humidiphila*" sensu epitype of Pažoutová & al. 2015, non Tanda 1979.]

Type—Germany, Bavaria, Philippsreut, 48.8564°N 13.6763°E, on *Dactylis* sp., 1988, Pažoutová (**Holotype**, PRM922708 [dried culture on T2 media]; ex-type culture CCC434; GenBank JX083704, JX083635).

= *Claviceps purpurea* var. *alopecuri* Tanda, J. Agric. Sci. (Tokyo) 22: 295 (1977).

= *Claviceps purpurea* var. *dactylidis* Tanda, J. Agric. Sci. (Tokyo) 25: 266 (1980).

Differs from *C. huimidiphila* in amplified TEF1-α region (918 nts) by at least seven sites (*C. humidiphila*/*C. bavariensis* = 271 A/G; 348 A/G; 642 T/C; 675 T/C; 688 C/T; 690 T/C; 860 T/C) that are identical within species.

Etymology—referring to the provenance of the type specimen

Sclerotia purplish brown (#452D2E), dark brown (#2D1E1E) to black (#272424), 3–10 × 0.3–1.4(–1.7) mm, ovoid, obclavate, narrow cylindrical,

Fig. 3. *Claviceps bavariensis*. A. Sclerotia on *Dactylis glomerata* (TNS-F-60477); B. Sclerotia on *Poa annua* (TNS-F-60470); C. Sclerotia on *Alopecurus aequalis* var. *amurensis* (TNS-F-60473); D. Sclerotia on *Festuca rubra* (DAOM 984880); E. Sclerotia on *Phalaris arundinacea* (DAOM984879); F. Sclerotia detached from PRM922708 (holotype); G. Sclerotia detached from DAOM 984880; H. Sclerotia detached from DAOM 984879; I. Longitudinal section near the rind showing textura prismatica (DAOM 984879); J, K. Ascostromata developed from germinated sclerotia from *D. glomerata* (TNS-F-60523), and *A. aequalis* var. *amurensis* (TNS-F-60521); L, M. Cross sections of sclerotia showing the color of inner tissue (DAOM 984879, 984880); N. Asci showing the thick caps with a central pore (TNS-F-60522); O. Conidiogenesis in sclerotia (DAOM 984879, 984880); P. Conidia washed off sclerotia (PRM922708, holotype); Q. Filiform ascospores (TNS-F-60520). Scale bars: A = 5 mm; B, C, F–H, J, K = 2 mm; D, E = 1 mm; L, M = 200 µm; I = 20 µm; N–Q = 10 µm.

curved, or subulate, floating in fresh water, interior white, greyish yellow (#D0CBA0) to olive brown (#4A423B) from center to margin, or vivid yellow (#FEE302) to deep yellow (#FFC40C) in center (3A8–4A8). Conidiogenous cell cylindrical, or obovoid, 12–23(–27) × 5–7 µm. Conidia ovoid, reniform, cylindrical, (5.5–)6.1–13.1(–14.3) × (2.3–)2.4–3.9(–4.4) µm, L/W ratio 2.1–2.2.

Ascostromata pinkish (#FFC0CB) to light brown (#5D4037). Stipe filiform 5–8 mm long. Capitellum 0.5–1 mm diam. Asci cylindrical, 50–155 × 2.8–5.0 µm. Ascospores filiform.

Additional specimens examined—**CANADA**, **Alberta**, **Beaverlodge**, on *Festuca rubra*, Aug. 2019, Henry Klein-Gebbinck (DAOM 984880; GenBank MW411019, MW411021), on *Phalaris arundinacea*, Aug. 2019, Henry Klein-Gebbinck (DAOM 984879; GenBank MW411018, MW411020). **JAPAN**, **Chiba**, **Sakura**, Usuiden, 35.7416°N 140.1850°E, on *Dactylis glomerata*, Jul 4, 2016, Kazuhito Tanada (TNS-F-60490; MAFF 247304; GenBank LC598969, LC598998); **Ishikawa**, **Hakusan**, Shiramine, 36.1150°N 136.6994°E, on *Poa acroleuca*, Jul 1, 2016, Eiji Tanaka (TNS-F-60489; GenBank LC598981, LC599010); Yorishinbo, 36.4770°N 136.5647°E, on *Poa annua*, Apr 29, 2016, Eiji Tanaka (TNS-F-60470; GenBank LC598977, LC599006); **Kanazawa**, Minato, 36.6450°N 136.6631°E, on *Calamagrostis epigejos*, Jul 18, 2016, Eiji Tanaka (TNS-F-60493; MAFF 247306; GenBank LC598985, LC599014); **Komatsu**, Imaemachi, 36.3772°N 136.4492°E, on *Poa trivialis*, May 9, 2016, Eiji Tanaka (TNS-F-60472; GenBank LC598979, LC599008); **Nomi**, Suedera, 36.4442°N 136.5081°E, on *Poa pratensis*, June 9, 2017, Eiji Tanaka (TNS-F-60504; GenBank LC598976, LC599005); **Nonoichi**, Nakabayashi, 36.5064°N 136.5994°E, on *Polypogon fugax*, June 5, 2017, Eiji Tanaka (TNS-F-60503; GenBank LC598986, LC599015); Suematsu, 36.5103°N 136.5967°E, on *Alopecuru. aequalis* var. *amurensis*, May 23, 2016, Eiji Tanaka (TNS-F-60473; culture MAFF 247299; GenBank LC598974, LC599003); (TNS-F-60521, fruiting bodies derived from TNS-F-60473); **Tsubata**, 36.6875°N 136.7589°E, on *F. rubra*, Jul 24, 2016, Eiji Tanaka (TNS-F-60497; GenBank LC598984, LC599013); **Uchinada**, Kose, 36.6758°N 136.6706°E, on *D. glomerata*, June 18, 2016, Eiji Tanaka (TNS-F-60477; GenBank LC598970, LC598999); (TNS-F-60523, fruiting bodies derived from TNS-F-60477); Koyodai, 36.6375°N 136.6300°E, on *Agrostis gigantea*, Jul 24, 2016, Eiji Tanaka (TNS-F-60495; GenBank LC598982, LC599011); **Iwate**, **Miyako**, Kuzakai, 39.6503°N 141.3574°E, on *D. glomerata*, Aug 7, 2019, Eiji Tanaka (TNS-F-60518; GenBank LC598973, LC599002); 39.6512°N 141.3574°E, on *Phleum pratense*, Aug 7, 2019, Eiji Tanaka (TNS-F-60516; GenBank LC598983, LC599012); **Kanagawa**, **Atsugi**, Nurumizu, 35.4347°N 139.3503°E, on *P. annua*, June 16, 2017, Kazuhito Tanada (TNS-F-60505; GenBank LC598978, LC599007); **Niigata**, **Sanjo**, Kamioura, 37.5883°N 139.0124°E, on *A. aequalis* var. *amurensis*, July 3, 2017, Kazuhito Tanada (TNS-F-60508; GenBank LC598975, LC599004); **Tokamachi**, Matsunoyama, 37.1189°N 138.5908°E, on *D. glomerata*, June 24, 2016, Eiji Tanaka (TNS-F-60481; GenBank LC598972, LC599001); 37.0885°N 138.6097°E, on *Poa palustris*, June 24, 2016, Eiji Tanaka (TNS-F-60482; GenBank LC598980, LC599009); **Tochigi**, **Tochigi**, Fujioka, 36.2436°N 136.6614°E, on *D. glomerata*, Jul 6, 2016, Kazuhito Tanada (TNS-F-60491; GenBank LC598971, LC599000).

Hosts—*Alopecurus*, *Agrostis*, *Ammophila*, *Calamagrostis*, *Dactylis*, *Deschampsia*, *Festuca*, *Molinia*, *Phalaris*, *Phleum*, *Phragmites*, *Poa*, *Polypogon*

Comments—In our phylogenetic tree (Fig. 1), two samples on *Alopecurus,* four on *Dactylis,* six on *Poa,* and one on *Polypogon* group in the *Claviceps bavariensis* clade, suggesting that Tanda's two varieties—*C. purpurea* var. *alopecuri* (Tanda 1977), *C. purpurea* var. *dactylidis* (Tanda 1981)—and the samples from *Poa* and *Polypogon* (Tanda 1980; Tanda & Kawatani 1980) might represent this species. In that case, a species name, "*C. alopecuri*" stat. nov., could be coined. However we considered the following: 1) the type specimen of *C. purpurea* var. *alopecuri* designated by Tanda (1977) was lost due to the same reason as *C. purpurea* var. *phalaridis*, therefore it is not possible to verify molecularly that the lost type truly belonged to the clade of *C. bavariensis,* which is essential for species delimitation; 2) the two specimens on *Alopecurus* in this study were not from Suginami, Tokyo, Japan (the type location); 3) the name "*C. alopecuri*" might give the false impression that the species has a host range limited to *Alopecurus*, contrary to the wide host range of the species. Therefore, we avoided elevating the varietal name to species level, proposing instead a new species name, *C. bavariensis*. Normally an epitype can only be replaced via conservation, but in this case the holotype specimen to which the epitype specimen would be attached has been lost, and an epitype no longer has nomenclatural status (Turland & al. 2018, Art. 9.20 Note 8). This allows us to use PRM922708, the epitype for *C. humidiphila* Pažoutová & M. Kolařík, as a holotype for *C. bavariensis*.

Tanda (1977, 1980, 1981) and Tanda & Kawatani (1980) recorded detailed sexual and asexual morphological features of samples from *Alopecurus, Dactylis, Poa,* and *Polypogon*, which could represent *Claviceps bavariensis*. In general, sclerotia are cylindrical with pointed or blunt ends; stromatal stipes are reddish brown, cylindrical or filiform, and curved or straight; capitella are light orange, oblate globose, and verrucose; perithecia are pyriform or ovoid; asci are narrowly cylindrical with thickened caps; ascospores are colorless and filiform; and the conidia are 1-celled, hyaline, and cylindrical or ovoid. The sizes of these characters sclerotia, stromata, perithecia, asci, ascospores and conidia varied significantly from different host plants (Table 1). Pažoutová & al. (2015) also provided more detailed morphological description and comparison with other species for *C. bavariensis* (as *C. humidiphila*). The conidia of *C. bavariensis* tend to be larger than *C. purpurea* s.str. and

Table 1. *Claviceps bavariensis:* observations cited in Tanda (1977; 1980; 1981) and Tanda & Kawatani (1980)

A—Host, sclerotial & ascomal dimensions

Host	Sclerotia (mm)	Stipe (mm)	Capitella* height × diameter (mm)
Alopecurus	1.5–5.1 × 0.3–0.9	1.0–4.0 × 0.3–0.5	0.4–0.9 × 0.6–1.3
Dactylis	3.2–11.7 × 0.6–1.7	1.0–14 × 0.1–1.4	0.3–1.5 × 0.3–2.3
Poa	1.5–5.8 × 0.2–1.5	1.0–9.0 × 0.1–0.7	0.4–1.0 × 0.5–1.9
Polypogon	1.2–4.0 × 0.2–1.0	1.0–7.0 × 0.2–0.6	0.4–1.1 × 0.5–1.4

B—Anatomical dimensions

Perithecia (µm)	Asci (µm)	Ascospore length (µm)	Conidia (µm)	Conidia L/W
151–221 × 77–144	75–138 × 2.5–3.7	74–126	5.0–11.3 × 2.6–5.0	2.1–2.3
154–270 × 88–168	81–151 × 1.8–4.2	74–119	4.0–14.8 × 1.8–4.7	1.9–2.8
147–256 × 60–154	77–158 × 1.8–5.3	63–151	3.2–12.6 × 1.6–5.8	2.0–3.0
119–210 × 56–126	91–133 × 2.5–3.9	84–123	5.0–12.8 × 2.4–5.4	—

C. arundinis, although the size ranges can overlap. Although these three species shared several host genera, population genetic analyses suggested significant genetic differentiation and limited gene flow among them (Pažoutová & al. 2015). The three species also differ in their ergot alkaloid spectra (Pažoutová & al. 2000, Negård & al. 2015) and ergochrome pigments (Flieger & al. 2019). Nevertheless, separation between *C. bavariensis* and *C. humidiphila* relies essentially on molecular evidence (mainly TEF1-α, at least seven constant nucleotide variance). The host species cited by Pažoutová & al. (2015) included 10 genera mainly from Europe (Belgium, Czech Republic, Germany, France, Norway, Poland, Turkey), but also from Central Asia (Kazakhstan, one specimen on *P. arundinacea*) and North America (USA, one specimen on *Ammophila breviligulata*). The present study expanded sample locations and host range by adding Canada (on *Festuca*, *Phalaris*) and Japan (on *Agrostis gigantea*, *Alopecurus aequalis* var. *amurensis*, *Calamagrostis epigejos*, *Dactylis glomerata*, *Festuca rubra*, *Poa acroleuca*, *P. annua*, *P. palustris*, *P. pratensis*, *P. trivialis*, *Polypogon fugax*).

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